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Training of a dog for the monitoring of *Osmoderma eremita*

Fabio Mosconi[1,2], Alessandro Campanaro[1,3], Giuseppe Maria Carpaneto[4], Stefano Chiari[1,4], Sönke Hardersen[3], Emiliano Mancini[3], Emanuela Maurizi[1,3], Simone Sabatelli[2], Agnese Zauli[1,3], Franco Mason[3], Paolo Audisio[2]

1 *Consiglio per la ricerca in agricoltura e l'analisi dell'economia agraria – Centro di ricerca Difesa e Certificazione, Via di Lanciola 12/a, 50125 Cascine del Riccio, FI, Italia* **2** *Università di Roma "La Sapienza", Dipartimento di Biologia e Biotecnologie "Charles Darwin", Via A. Borelli 50, 00161 Roma, Italia* **3** *Centro Nazionale per lo Studio e la Conservazione della Biodiversità Forestale "Bosco Fontana" – Laboratorio Nazionale Invertebrati (Lanabit). Carabinieri. Via Carlo Ederle 16a, 37126 Verona, Italia* **4** *Università Roma Tre, Dipartimento di Scienze, Viale Guglielmo Marconi 446, 00146 Roma, Italia*

Corresponding author: *Fabio Mosconi* (fabio.mosconi@gmail.com)

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Abstract

One aim of the MIPP Project (http://www.lifemipp.eu) was to develop non-invasive monitoring methods for selected saproxylic beetles. In this paper, a method is proposed for monitoring the larvae of *Osmoderma eremita* in their natural habitat (i.e. hollow trees), using a conservation detection dog (CDD). Wood mould sampling (WMS), the standard method to detect hermit beetles and other saproxylic insects inside tree hollows, is time-consuming and exposes the target species and the whole saproxylic communities to some risks. In contrast, CDDs pose no risk to the species living inside trees while, at the same time, offer a powerful tool for surveying the insects. In this paper, the methods applied to train the dog are presented, together with the results for accuracy (the overall proportion of correct indications), sensitivity (the proportion of correct positive indications) and specificity (the proportion of correct negative indications) obtained once the CDD had been fully trained. Results are presented for nitrocellulose filters with the odour of the target species, for larvae living inside hollow trees, for frass and for the remains of adults. A comparison of the efficiency between CDD and WMS showed that employing the dog was much less time-consuming than WMS.

The literature on training CDDs for nature conservation tasks, with particular reference to cases involving Coleoptera, was also reviewed.

Keywords
Conservation detection dog, *Osmoderma eremita*, saproxylic beetles, Habitats Directive, monitoring methods, conservation biology

Introduction

In the last few decades, conservation detection dogs (CDDs) (Beebe et al. 2016) have been increasingly used for the protection of wildlife due to their keen sense of smell for locating biological targets. These skills offered by dogs for the collection of wildlife data are well recognised and documented in recent reviews (Helton 2009, Dahlgren et al. 2012, Johnen et al. 2013, Beebe et al. 2016). The first case of dogs employed in nature conservation dates back to the 1890s in New Zealand when they were used to find endangered bird species: the kiwi (*Apteryx* sp.) and the kakapo (*Strigops habroptila* Gray, 1845) (Hurt and Smith 2009, Bebee et al. 2016). At present, the use of CDDs is widespread throughout the world for assisting in an array of activities, including detection of wildlife (plants and animals), carcasses of birds and bats, scats, pathogens and other biological materials (Bebee et al. 2016). In the majority of projects, dogs were used for the detection of scats, while direct searching for wildlife is the second most important use and, only in a minority of cases, carcasses were detected (Bebee et al. 2016). The two main reasons why species are being searched for are: i) they are rare and endangered and ii) they are alien species. In the management of endangered and threatened species, dogs are mostly used to search for mammals (Wasser et al. 2004, Browne et al. 2006, Hurt and Smith 2009, Long et al. 2007, Coppolillo et al. 2015), birds (Browne et al. 2006, Hurt and Smith 2009) and reptiles (Browne 2005, Cablkand Heaton 2006, Cablk et al. 2008, Nussear et al. 2008, Hurt and Smith 2009). Dogs have been used to search for accidentally imported alien species particularly focusing on reptiles (Savidge et al. 2011), rodents (Gsell et al. 2010) and nematodes (Richards et al. 2008). Conservation dogs are also employed to detect the occurrence of birds and bats killed by turbines in order to evaluate the impact of wind farms on wildlife (Arnett 2006, Paula et. al 2011).

Concerning invertebrates, pest, alien and invasive insect species are the main targets and indeed, conservation dogs were used for the first time to detect the gypsy moth *Lymantria dispar* (Linnaeus, 1758) (Wallner and Ellis 1976). Since then, many species belonging to different insect orders have been searched by dogs: Coleoptera (Nakash et al. 2011, Errico 2012, Hoyer-Tomiczek and Sauseng 2013, Kelley 2013, Hoffman 2014, Suma et al. 2014, Coppolillo et al. 2015, Hoyer-Tomiczek et al. 2016), Diptera (Welch 1990), Hemiptera (Pfiester et al. 2008, Rolón et al. 2011), Hymenoptera (Lin et al. 2011, Brocos and González 2015), Isoptera (Brooks et al. 2003, Zahid et al. 2012) and Lepidoptera (Wallner and Ellis 1976). So far, dogs have been used in a limited number of projects to locate protected or endangered insects, such as bumblebees (Waters et al. 2011, O'Connor et al. 2012).

In the LIFE Project MIPP (Monitoring of Insects with Public Participation, www.lifemipp.eu, Action A.4: "Acquisition and training of Osmodog"), a CDD was trained to find the larval stages of the hermit beetle *Osmoderma eremita* (Scopoli, 1763) in its natural habitat (see Mason et al. 2015 and Carpaneto et al. 2017). *O. eremita* is an endangered saproxylic beetle protected in the European Union by the Bern Convention and listed in annexes II and IV of the Habitats Directive 92/43/EEC and also reported as "Near Threatened" in IUCN Red Lists of Threatened Species (Nieto et al. 2009, Nieto and Alexander 2010). This species is associated with old hollow broad-leaved trees in mature forest ecosystems, as well as outside closed forests (e.g. pasture woodlands) (Ranius et al. 2005, Giangregorio et al. 2015, Maurizi et al. 2017).

The CDD of the MIPP project is the first case of a dog searching for an endangered beetle species, *O. eremita*. Indeed, a special feature of conservation dogs is that they can detect cryptic and/or elusive species (Hurt and Smith 2009) and this makes them excellent tools to locate insects. In general, the use of dogs in nature conservation offers many advantages. For example, it can facilitate the location of target species in unreachable habitats (Chambers et al. 2015, Hoyer-Tomiczek et al. 2016), thus decreasing the risk of disturbance and performing non-destructive sampling (Chambers et al. 2015), with particular reference to saproxylic species with larvae living inside hollow trees (Hoyer-Tomiczek et al. 2016). Moreover, the use of dogs minimises the sampling effort, in terms of number of personnel involved in fieldwork and time spent in the field (Browne et al. 2006, Harrison 2006, Duggan et al. 2011, Paula et al. 2011) and reduces the bias in sampling (Wasser et al. 2004, Browne et al. 2006). All of these characteristics fit well with the *O. eremita* study case. Indeed, without the aid of a dog, most of the sampling methods commonly used to detect (and monitor) the hermit beetles involve wood mould sampling (Ranius et al. 2005, Chiari et al. 2014) and passive or active traps (Ranius 2001, Svensson and Larsson 2008, Chiari et al. 2013, Maurizi et al. 2017), which are characterised by the disadvantages listed above. Locating larvae inside trunks using wood mould sampling is the main method which allows the identification of breeding sites of hermit beetles and other saproxylic insects. It consists of digging inside tree cavities to find specimens or their remains. However, it is not often possible with wood mould sampling to investigate all cavities of a tree; in particular, cavities located high up or with narrow openings are very difficult to explore. A further drawback of this method is the physical risk for the target species and for the whole saproxylic community living inside the trunk (Tikkamäki and Komonen 2011, Chiari et al. 2014). Last, but not least, a dog-handler team only requires a few minutes to analyse a single tree, while wood mould sampling is a time-consuming technique (Chiari et al. 2014).

Searching for larvae has some additional advantages as adults have a short period of activity (Maurizi et al. 2017) and population size of adults fluctuates during the activity period and in correlation with environmental factors. In contrast, larvae are always present inside hollow trees during their development which lasts about 3 years. In this paper, a summary of the training techniques for dogs employed in nature conservation, with particular reference to the cases involving Coleoptera is presented. The information obtained by this literature review was used to plan and perfect the training strategy for the dog.

Dog breed and training

The choice of the right dog is critical to the success in the training for finding insects. First of all, a dog must have a suitable *drive* for the specific task required and these motivational characteristics differ between breeds. As indicated by Cablk and Heaton (2006), choosing a breed with a mix of *hunt drive* and *play drive* would be preferable for a CDD. In general, pure-bred dogs are preferred to mixed breeds, as the traits of mixed dogs could be unpredictable (Dahlgren et al. 2012). Recently, it has been shown that breeds that had been originally and specifically selected for scent work (e.g. Beagle, German Pointer, Bracco Italiano), do, in fact, demonstrate a higher olfactory acuity than breeds that had not been selected for such purpose (e.g. English greyhound, Siberian husky) (Polgár et al. 2016). Furthermore, it would be preferable to choose a dog from a specific line that has already shown traits and skills suitable for the specific work. Finally, individual traits are very important in a given breed: dogs can differ in physical and psychological traits (e.g. agility, physical fitness, various aspects of intelligence, predisposition to collaboration and curiosity), all of which can strongly influence the quality of work (Dahlgren et al. 2012). In previous studies, the breed most used in searches for Coleoptera were Golden Retriever (Nakash et al. 2000, Suma et al. 2014), Labrador Retriever (Errico 2012, Coppolillo et al. 2015) and Border Collie (Hoffman 2014, Hoyer-Tomiczek et al. 2016).

It is preferable to use adult dogs for the fieldwork as juvenile dogs may have lower levels of attention and concentration than adults (Hurt and Smith 2009). In addition, adult dogs can perform research routines better than juvenile dogs (Suma et al. 2014). Hurt and Smith (2009) suggest that dogs can be ready to work when they are between 12 and 24 months old. However, the training can start earlier (Dahlgren et al. 2012). In particular, before starting the actual training, the dog needs to undertake base obedience training (Welch 1990, Richards et al. 2008) and needs to be introduced to the search routines through easy search games by encouraging the dog to find toys or other fun targets (Dahlgren et al. 2012).

Living targets, in this case insect larvae, have an odour which is characteristic for each species and specific for the environment in which they live. This is a very important parameter to consider when choosing the scent target to use for the training. Indeed, the saproxylophagous and xylophagous species, living inside wood mould or creating galleries in trunks, have a broad scent bouquet due to different sources of odours present in their habitat (e.g. fungi, sawdust and other organic materials) (Hoyer-Tomiczek et al. 2016). For this reason, as the target for the dog, the training routines include living specimens as well as material from their natural habitat and the training must proceed by presenting odours of increasing complexity (Errico 2012, Suma et al. 2014, Hoyer-Tomiczek et al. 2016). After the first target, a dog can learn to recognise more odours (see below) and, in some cases, more than 20 odours can be recognised (Long et al. 2007, Coppolillo et al. 2015).

The method to train conservation dogs in finding live animals is similar to those used to find unanimated and non-biological targets and it is based on the positive re-

inforcement of the dog's behaviour (Hurt and Smith 2009, Braun 2013, Johnen et al. 2013). The method is built on rewarding the dog with food or play as the primary reinforcement, immediately after correct signalling. It was used in all studies in which dogs detected Coleoptera (Nakash et al. 2011, Errico 2012, Kelley 2013, Hoffman 2014, Suma et al. 2014, Hoyer-Tomiczek et al. 2016). Clickers, devices that emit a double-click sound (Smith and Davis 2008), can be used as positive secondary reinforcement when the trainer is distant from the dog and cannot give the primary reinforcement immediately after correct signalling (Braun 2013). Although the clicker is mainly a predictor for food (Johnen et al. 2013), it has other complex implications for the dog (Smith and Davis 2008). Training with positive reinforcement should be a progressive process. During the first phase of the training, the dog must be "imprinted" on the target odour (Hoyer-Tomiczek et al. 2016); in practice, the dog must learn that the recognition of a specific odour is linked to a reward. In this context, *imprinting* has the meaning of "teaching odour discrimination skills to a dog" as reported by Fjellanger (2003). The imprinting starts when the dog makes, for the first time, an olfactory contact with the target odour and immediately receives the reward. This scent detection routine should be repeated until the dog has learned clearly and unambiguously how to find and indicate its target (Hoyer-Tomiczek et al. 2016). The target can be presented to the dog *naked* or inside a small perforated box.

During the second phase of training, the scent discrimination phase, the dog learns to discriminate between the target odour and other scents by consolidating, at the same time, the search behaviour and the signalling display; this process is also called "generalisation" of an odour (Hurt and Smith 2009). The generalisation can be stimulated by offering to the dog simultaneously the target odour and other scenting materials and rewarding only after correct signalling. Several kinds of settings can be arranged for the training: the scenting target and non-target material can be presented simultaneously to the dog or one or more targets can be hidden in natural settings or in fenced training areas (Braun 2013). Although the training area must have similar characteristics to those where the real research work will be carried out, the area must be free of the target to avoid confusing the dog (Errico 2012). During the discrimination phase, it is very important to correctly build the communication between the dog and the handler, to develop the dog's search behaviour and to reinforce the focus on the target odour (Hurt and Smith 2009). All search routines and behaviour must be shaped and reinforced by the trainer, by encouraging the dog until it can perform long search sessions without loss of attention (Johnen et al. 2013, Hoyer-Tomiczek et al. 2016). To activate the dog's search behaviour, a specific word from the handler (e.g. *search* or *find it*) must be associated to the specific work that the dog has to perform (Wallner and Ellis 1976, Welch 1990, Rolón et al. 2011, Hoyer-Tomiczek et al. 2016).

Johnen et al. (2013) suggest that the duration of training for sniffer dogs can vary between 7 days and 16 months, but for CDD this period may be longer, as searching for biological targets in nature is likely to be complicated by the presence of numerous olfactory stimuli or by the smell of species related to the target species (Wallner

and Ellis 1976, Hurt and Smith 2009). The basic training period (imprint phase) for inexperienced dogs searching for insects is between 1 to 3 months and the subsequent discrimination phase lasts for a further 6 to 7 months (Wallner and Ellis 1976, Suma et al. 2014, Hoyer-Tomiczek et al. 2016). Dogs that have already been trained to search for biological targets and in fieldwork need less time to be ready to work (Brooks et al. 2003, Lin et al. 2011). The total length of training is related to numerous factors, such as the trainer's experience, the trainer's skills, the characteristics of the breed and the traits of individual dogs (Hurt and Smith 2009, Johnen et al. 2013). The weekly rate of training and the daily length of the working sessions can vary in function from the level of training, the familiarity with fieldwork and the skills of the trainer. Different authors indicate a rate from 3 to 5 training sessions per week; each session can last from 2 to 4 hours with breaks related to the level of fatigue in the dog and to temperature (Harrison 2006, Hurt and Smith 2009, Lin et al. 2011, Suma et al. 2014).

A dog can be trained to offer an active or passive response to the target odour (Braun 2013). A passive response consists of pointing with its nose towards the target and/or stopping and sitting close to the scent source (Long et al. 2007). This response is preferable in cases in which the dog might potentially cause disturbance or frighten the target species and also in cases in which the dog might be at risk (by aggressive or dangerous species) (Braun 2013). Active signalling includes various reinforcing behaviour in addition to pointing, such as scratching, barking or sitting and looking at the scent source and handler (Hoyer-Tomiczek et al. 2016). If needed, all these behavioural activities can be enhanced and shaped by delaying the reward after correct signalling (Johnen et al. 2013, Hoyer-Tomiczek et al. 2016). In general, an active response is suitable for conservation dogs searching for beetles.

Creating a cohesive and efficient dog-handler team is critical to the success of the work; the dog must be able to search, locate and signal the target, while the handler has to make this possible by managing the dog in the field and, at the same time, correctly interpreting the behaviour of the dog. It is important to recognise that the searching ability varies between different dog-handler teams (Johnen et al. 2013, Hoyer-Tomiczek et al. 2016). In particular, the handler must: 1) motivate and handle the dog during the work; 2) create a relationship of trust with the dog and maintain a relaxed atmosphere during each search session; 3) understand the dog's searching behaviour and its reactions in the presence of a possible target, as the dog's reactions may depend on several factors such as its psychophysical conditions or weather conditions (Long et al. 2007, Hurt and Smith 2009, Dahlgren et al. 2012); 4) reward the dog adequately after correct signalling, as each activity in the field is simultaneously a training session for it and 5) take care of the dog during the field search, taking into account its needs and other factors that may cause fatigue and loss of concentration, such as thirst, long work sessions on steep terrain or adverse weather conditions that could compromise the quality of work (Dahlgren et al. 2012).

Several authors suggest that the best parameters to describe the ability of conservation dogs in correctly locating their target are the overall percentage of correct indications and the percentage of correctly detected targets for the total number of

targets (Wallner and Ellis 1976,Welch 1990, Engeman et al. 2002, Brooks et al. 2003, Cablk and Heaton 2006, Long et al. 2007, Richards et al. 2008, Gsell et al. 2010, Lin et al. 2011, Waters et al. 2011, Suma et al. 2014, Hoyer-Tomiczek et al. 2016). Although these parameters are often characterised by different names, for this work, the definition used by Allouche et al. (2006) was adopted in relation to the assessment of presence-absence predictive models i.e. accuracy (the overall proportion of correct indications), sensitivity (the proportion of correct positive indications) and specificity (the proportion of correct negative indications).

In the majority of the previously listed cases, the average accuracy for conservation dogs was around 90%, although it was variable. Low accuracy may be caused by several factors, such as: age of the dog, insufficient training, problems in the dog-handler team communication, inexperience of the trainer and/or handler (Savidge et al. 2011, Johnen et al. 2013). Exercise and training can increase the accuracy of the dog: dog-handler teams with more experience have a higher accuracy (Savidge et al. 2011). Moreover, dogs employed in new environments have initially low accuracy although this can be increased with training (Wallner and Ellis 1976).

While working in the field, several sources of disturbance can further decrease the accuracy of the search: temperature, wind, fatigue and presence of wild animals, their traces or humans (Hurt and Smith 2009, Dahlgren et al. 2012, Hoyer-Tomiczek et al. 2016). It is important to consider that the accuracy can be artificially increased by the handler; in fact, a handler who knows the hiding places of the targets can unconsciously give this information to the dog (Clever Hans effect) or the dog can follow the odour trail made by the trainer while hiding the target (Lin et al. 2011, Johnen et al. 2013). For this reason, accuracy must to be assessed with a "double blind test", in which the location of the targets is unknown to both the dog and the handler and special precautions must be taken to avoid helping the dog to find its target (Brooks et al. 2003, Cablk and Heaton 2006, Johnen et al. 2013, Hoyer-Tomiczek et al. 2016).

Methods

Breed choice and training methods

The breed of dog selected as a CDD suitable for searching *O. eremita* was the Golden Retriever, a breed widely used to search for biological targets. In fact, the olfactory capabilities of these dogs and their nature make them easy to train and to handle during fieldwork. The dog, which was named Teseo (Figure 1), was chosen from a specific line from which many individuals have been employed as CDDs to find illegally imported animals and animal parts (CITES Service for the Comando Unità Tutela Forestale Ambientale e Agroalimentare Carabinieri – CUTFAA, the former Italian State Forestry Corps). Teseo started working with its trainer/handler at the age of 6 months and the actual fieldwork being carried out once the dog had reached adulthood (24 months), at the time when the trainer considered the dog to be well trained.

Figure 1. Teseo wearing the harness (Photo by Fabio Mosconi).

The training was carried out in successive steps, as a function of the age of the dog and according to the level of skill (Table 1). A collection permit was issued by the Ministero dell'Ambiente e della Tutela del Territorio e del Mare - DG Protezione della Natura e del Mare (U.prot PNM 2012-0010890 del 28/05/2012) to handle larvae of *O. eremita* for dog training.

Age 6–9 months: base training

During the early months of the dog's life, preparatory activities for the next training steps were carried out: i) basic obedience training, in which the dog learned some basic

Table 1. Summary of the training progression of Teseo.

Dog age (months)	Location	Training	Rate
6–9	Fenced training area and public parks	Basic obedience training Search games Agility activities	2 to 3 times/week
9–16	Fenced area	Imprinting phase (target: living larvae)	2 to 5 times/week
16–24	Natural areas *Osmoderma*-free	Discrimination phase (target: nitrocellulose filters with the smell of the target) Preliminary discrimination tests (target: living larvae of *Oryctes nasicornis*, *Gnorimus variabilis*)	5 times/week

commands (e.g. stay, come, sit etc.); ii) search games, i.e. hiding small toys or pieces of food and rewarding the dog with play and iii) agility activities, in order to improve the oral and gestural communication between the dog and handler. During this phase, 2 or 3 training sessions were performed each week in a fenced area. Each session lasted for no more than 2 hours with several breaks to avoid stressing the puppy.

Age 9–16 months: imprinting phase

From this phase onwards, the dog wore a harness when working (Figure 1), both during training and fieldwork. Additionally, the dog was conditioned with a clicker and a special word (i.e. "search") was associated with starting the searches. To imprint the dog to the target odour, some *O. eremita* larvae were used. These larvae were kept in boxes filled with the wood mould collected from their natural habitat. During the initial trials, the larva was washed with water and kept in a perforated box. Simultaneously, empty boxes were presented as control. The washing was necessary to ensure that the dog was imprinted with the pure smell of the target by eliminating other odours related to the specific microhabitat in which the larvae live. The dog was rewarded with the clicker, praise and small pieces of food only when coming into contact with the target odour and when showing a reaction. When the dog had learned to recognise the target odour, the training continued by hiding the washed larva in places easy to find for the dog (i.e., inside basal cavities of trees, under leaves on the ground or under bark), within a fenced training area. Disposable latex gloves were used when handling larvae, to avoid transferring the target odour to the hands of the trainer. This training phase ended when Teseo had successfully learned to locate and signal the target to the trainer. At a later date, the signalling by the dog was shaped and reinforced by delaying the reward as described by Hoyer-Tomiczek et al. (2016) until Teseo had learned proper signalling behaviour.

In the following phases, the larvae were placed inside perforated vials without washing in order to better protect them during the work (Figure 2). This allows positioning the target deep inside cavities without the risk of losing the larvae and the dog can learn the scent bouquet of the larvae (i.e. the scent of the larva plus odours of

Figure 2. Perforated vials in which the larvae were inserted to protect them during training (Photo by Fabio Mosconi).

wood mould). During each training session, the dog searched one tree a time and was rewarded only when it signalled the correct tree.

During every training day, 1 to 5 consecutive sessions were carried out with a single target hidden in a tree and this was repeated for 2 to 5 times a week. The number and the length of the daily working sessions were gradually increased as was the number of trees without the target. After 1 or 2 sessions, the dog was allowed to rest and to play for 5–15 minutes. The imprinting phase ended when the dog had learned to search and unambiguously signal the target.

Age 16–24 months: discrimination phase

During the discrimination phase, the training sessions were carried out as simulations of real fieldwork. The training was conducted in natural and semi-natural areas suitable for *O. eremita* (Table 2). In some areas, a number of independent sub-areas were de-

Table 2. Summary of the areas in the province of Rome (Latium, Italy) where the training and the accuracy tests were carried out. SA: sub-areas for training sessions; TREES: tree species present in the area and in which the target was hidden (Qi: *Quercus ilex*; Qs: *Quercus suber*; Pt: *Populus tremula*); TR: training areas; AC: accuracy test areas; Oasi = private Nature Reserve; PRU = Regional Urban Park; RN = Nature Reserve; Villa = city park with annexed historical buildings.

Area	SA	TREES	TR	AC
RN Monte Mario	1	Qi; Qs	x	x
RN Monte Mario	2	Qi	x	
Villa Doria Pamphilj	1	Qi; Qs	x	x
Villa Doria Pamphilj	2	Qi; Qs	x	x
Villa Doria Pamphilj	3	Qi	x	x
Villa Doria Pamphilj	4	Qi; Qs	x	
PRU del Pineto	1	Qs	x	x
PRU del Pineto	2	Qs	x	x
PRU del Pineto	3	Pt	x	
RN Insugherata	1	Qi; Qs	x	
Oasi LIPU Castel di Guido	1	Qi	x	

fined to increase the number of training sites and these were used in a haphazard order to avoid familiarisation of the dog to individual areas. As far as it is known, *O. eremita* has never been reported from any training site used in the present research.

Small nitrocellulose filters were impregnated with the target odour by placing the larva in small containers, filled with filters for at least 8 hours. Filters, prepared in this way, can retain the target odour for a long period, if stored in hermetic containers. These filters are small and can be easily hidden in very small cavities and are thus invisible to the dog. These filters also allow the undertaking of long training sessions with multiple targets, simulating areas with a low or a high population density of *O. eremita*. A further important point in favour of the filters is the fact that the use of live larvae of the target species (i.e. a protected insect), can be substantially reduced. Disposable latex gloves and tweezers were used when handling filters to avoid transferring the target odour to the hands of the handler and field assistants.

In this phase, during each training session, 3 to 15 filters were hidden in randomly selected trees. To avoid the smell left behind by the trainer while placing the filters and which could influence the dog, all trees to be searched (those with and without target) had been touched by the trainer prior to the actual session. Only then was Teseo permitted to search one tree at a time, alternating between trees with and without targets (Figure 3). The dog was rewarded with the clicker, praise and small pieces of food every time it correctly signalled. In case of signalling a tree not containing the target, no reward was given and the handler simply led the dog to the next tree. Every training session always finished with finding the target. To this end, a filter was hidden inside a tree in a place which was easy to find. Once the final target had been found, Teseo was rewarded as usual and a few minutes were dedicated to play. The length and the complexity of the single sessions were gradually increased up to a maximum of 50

Figure 3. Teseo searching on a tree according to the indication of the handler (Photo by Emilia Capogna).

minutes of work with about 60 trees searched. During this phase, fieldwork lasted up to a maximum of 5 days per week, with 1 to 3 training sessions interspersed by 15–20 minutes of rest. Some double-blind training sessions were carried out with the help of a field assistant in order to avoid errors in the searches induced by the handler. The double-blind tests were also useful to assess when the dog had reached a level of training appropriate to work in a field study, i.e. when the dog, during a 50 minutes training session, stayed below a maximum error rate of about 15%.

Preliminary discrimination tests

Tests were carried out to verify whether Teseo misidentified larvae of species closely related to *O. eremita* and potentially syntopic in natural habitats. Some preliminary tests were carried out using larvae of *Oryctes* (*Oryctes*) *nasicornis* (Linnaeus, 1758) (Coleoptera: Scarabaeidae: Dynastinae) and *Gnorimus variabilis* (Linnaeus, 1758) (Coleoptera: Scarabaeidae: Cetoniinae). In every test, one larva of *O. eremita* was presented with one of the other species listed above. The larvae were hidden in perforated boxes and randomly mixed with empty boxes. Each day, 2 to 4 tests were carried out with a 10 minutes' break between two successive tests. The dog was rewarded only after signalling correctly.

Measurement of the accuracy

Accuracy, sensitivity and specificity were calculated following Allouche et al. (2006) as reported in Table 3, from data gathered in 8 wooded areas with two different approaches. A first set of tests was carried out in 6 sites without populations of *O. eremita* although they did contain hollow or fractured trees suitable for the larvae of this species (Table 4).

Table 3. Definition and formulae to calculate Accuracy, Sensitivity and Specificity. CPS: correct positive signalling (total n° of targets present in trees and correctly detected and signalled by the dog); CNS: correct negative signalling (total n° of trees without target and not signalled); NT: total n° of trees investigated; TT: total n° of targets present in the trees; TND: target not detected (total n° of targets present in the trees and not detected); NR: no reaction; TWD: targets wrongly detected (total n° of trees without target signalled by the dog as if the target was present).

Accuracy	the overall proportion of correct indications ("the rate of correct classification", Allouche 2006)	CPS+CNS/NT
Sensitivity	the proportion of correct positive indications ("the probability that the model will correctly classify a presence", Allouche 2006)	CPS/TT(CPS+TND)
Specificity	the proportion of correct negative indications ("the probability that the model will correctly classify an absence", Allouche, 2006)	CNS/NR(CNS+TWD)

Table 4. Areas (AREA) and sub-areas (SA) in the province of Rome where the accuracy tests with nitrocellulose filters were carried out. TREES: tree species where filters were hidden (Qi: *Quercus ilex*; Qs: *Quercus suber*); NT: total n°of trees investigated; TT: total number of targets. Indication by Teseo: NR: total n° of "no reaction", recognised as "target not present", PS: total n° of "partial signalling" recognised as "false signalling", CS: total n° of "complete signalling" recognised as "target detected"; Results: CPS: total n° of correct positive signalling (total n° of target correctly detected by the dog), TND: total n° of not-detected targets, CNS: correct negative signalling (NR to a tree without target), TWD: target wrongly detected (wrong CS to a tree without target); ACC: accuracy (CPS+CNS/NT); SEN: sensitivity (CPS/CPS+TND); SPE: specificity (CNS/CNS+TWD).

Area	SA	TREES	NT	TT	NR	PS	CS	CPS	TND	CNS	TWD	ACC	SEN	SPE
RN Monte Mario		Qi; Qs	49	6	44	3	5	4	2	42	1	93.87	66.66	97.67
Villa Doria Pamphilj	1	Qi; Qs	37	5	27	6	10	5	0	27	5	86.49	100	84.37
Villa Doria Pamphilj	2	Qi; Qs	46	6	36	8	10	6	0	36	4	91.30	100	90.00
Villa Doria Pamphilj	3	Qi	60	7	49	8	11	6	1	48	5	90.00	85.71	90.57
PRU del Pineto	1	Qs	20	4	16	2	4	4	0	16	0	100	100	100
PRU del Pineto	2	Qs	51	6	45	1	6	3	3	42	3	94.18	50.00	93.33
MEAN												92.64	83.73	92.66

Table 5. Accuracy measurements in Forcella Buana (FB) and San Vito (SV) considering larvae and frass and remains of adults (F+R) as target. NT: total number of trees investigated; TT: total number of targets present in trees; L13 and L16: total number of trees colonised by larvae of *O. eremita* in 2013 and 2016 respectively; F+R: total number of trees in which frass (F) and/or remains of adults (R) were found in 2013 and 2016; NR: no reaction; PS: partial signalling; CS: complete signalling; CPS: correct positive signalling; TND target non-detected; CNS: correct negative signalling; TWD: target wrongly detected.

		TT				**Indications by Teseo**			**CPS**				**TND**				**CNS**	**TWD**
Area	**NT**	**L13**	**L16**	**F+R**	**TOT**	**NR**	**PS**	**CS**	**L13**	**L16**	**F+R**	**TOT**	**L13**	**L16**	**F+R**	**TOT**		
FB	84	4	4	15	23	61	7	23	4	1	4	9	0	3	11	14	47	14
SV	48	2	2	6	10	32	8	16	1	2	4	7	1	0	2	3	29	9
TOT	132	6	6	21	33	93	15	39	5	3	8	16	1	3	13	17	76	23

The targets (filters with the target odour) were hidden by a field assistant unbeknown to dog and handler (double blind test) in randomly chosen trees. Before the search, the field assistant touched all trees to be searched to avoid providing olfactory help for the dog. During the search sessions, the handler reported to the field assistant the interpretation of the dog's behaviour, distinguishing between: no reactions (NR, the dog showed no reactions indicating the target); complete signalling (CS, the dog pointed to the target, sat, barked and looked at the scent source and handler). Sometimes the dog performed partial signalling (PS, e.g. the dog did not sit or barked only weakly). In these cases, the handler recognised the reaction as "false signalling" (FS) and reported that the target was not present (considered as "NR"). All results were recorded in a field sheet (Figure 4). The dog was rewarded only for correct signalling. Again, all sessions ended with finding the target.

<table>
<tr><th colspan="3">Session ID</th><th colspan="3">Date</th><th colspan="2">S/E Time</th></tr>
<tr><th colspan="6">Area</th><th colspan="2">Operators</th></tr>
<tr><td rowspan="2">Weather</td><td colspan="2">T(i)</td><td colspan="2">H(i)</td><td colspan="2">W(i)</td><td rowspan="2">Notes</td></tr>
<tr><td colspan="2">T(f)</td><td colspan="2">H(f)</td><td colspan="2">W(f)</td></tr>
<tr><td></td><td></td><td colspan="3">Dog indications</td><td colspan="2">Results</td><td></td></tr>
<tr><td>Tree ID</td><td>Trg</td><td>NR</td><td>PS</td><td>CS</td><td>TP</td><td>FS</td><td>Notes</td></tr>
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The accuracy of Teseo was also measured in two areas (San Vito and Forcella Buana) where the presence of *O eremita* had been ascertained in previous studies by wood mould sampling (Chiari et al. 2014). In 2013 and in 2016, the presence of the target species was recorded for single trees by the presence of larvae of *O. eremita*, frass or remains of adult specimens. Additionally, the presence of larvae of other flower chafer species was recorded. These data were used to perform a series of double blind tests. The dog-handler team searched individual trees and communicated the outcome to the field assistant (Figure 4). Teseo was only rewarded if the presence of *O. eremita* was indicated correctly.

The overall accuracy, sensitivity and specificity (Table 3) were calculated considering as "presence" of *O. eremita* in a tree: records of larvae in any year (2013 and 2016), records of frass and of remains of adults. Accuracy, sensitivity and specificity were calculated with and without considering records of frass and remains (Tables 5 and 6). Additionally, to test if the dog might also signal the presence of larvae of other species of flower chafers, sensitivity was calculated in a separate analysis, considering as "presence" any records of larvae of Cetoniinae in trees without *O. eremita,* as found previously by the wood mould sampling (Table 7). It was expected that Teseo would not recognise the larvae of the flower chafers as the target and, thus, it was expected to observe low values for sensitivity. All results were recorded in a field sheet (Figure 4). The contribution of the handler to the overall accuracy was calculated as the difference between the accuracy including the "partial signalling" as "no reaction" (after the correction of the handler) and the accuracy including the PS as "target wrongly detected" (Table 8).

The results of the evaluation of accuracy are summarised in Table 8.

Efficiency

The efficiency of the dog-handler team in detecting *O. eremita* larvae inside trees in Forcella Buana and San Vito was compared with the efficiency of the wood mould sampling technique in the same areas. The average time spent in 2016 to investigate a single tree by the two methods was calculated as the proportion between the total amount of time spent in the field by all operators and the total number of trees investigated. Two operators were needed to work with the dog and two operators were also required for wood mould sampling for each tree.

Results

Breed choice and training methods

At the end of the training period, Teseo was ready for the fieldwork; the dog was able to detect larvae of *O. eremita* and to signal the presence of its target inside trees by sitting

Figure 4. The CDD team: the dog, the handler and the field assistant ready for a working session (Photo by Emilia Capogna).

down, barking and looking at the scent source and the handler (Figure 5). Sometimes, Teseo scratched the bark if the source of the odour was located in the upper part of a tree. In a single field session, the dog was able to work for about 50 minutes, after which it needed to rest for 15 to 60 minutes before resuming work. Fatigue and high ambient temperatures caused a general decrease in the dog's working ability.

Table 6. Accuracy measurements in Forcella Buana (FB) and San Vito (SV) considering only larvae as target. NT: total number of trees investigated; TT: total number of target present in trees; L13 and L16: total number of trees colonised by larvae of *O. eremita* in 2013 and 2016 respectively; F+R: total number of trees in which frass (F) and/or remains of adults (R) were found in 2013 and 2016; NR: no reaction; PS: partial signalling; CS: complete signalling; CPS: correct positive signalling; TND targets non-detected; CNS: correct negative signalling; TWD: target wrongly detected.

		TT			Indication by Teseo			CPS			TND			CNS	TWD
Area	NT	L13	L16	TOT	NR	PS	CS	L13	L16	TOT	L13	L16	TOT		
FB	84	4	4	8	61	7	23	4	1	5	0	3	3	58	18
SV	48	2	2	4	32	8	16	1	2	3	1	0	1	31	13
TOT	132	6	6	12	93	15	39	5	3	8	1	3	4	89	31

Table 7. Accuracy measurement for larvae of flower chafers species. NT: total number of trees; TT: total number of targets. Indication by Teseo: NR: no reaction, PS: partial signalling, CS: complete signalling; Results: CPS: correct positive signalling, TND targets non-detected, CNS: correct negative signalling, TWD: target wrongly detected.

Area	NT	TT	NR	PS	CS	CPS	TND	CNS	TWD
Forcella Buana	84	32	60	6	24	7	25	35	17
San Vito	48	14	30	5	18	5	9	21	13
TOT	132	46	90	-	42	12	34	56	30

Table 8. Summary of Teseo's accuracy (ACC), sensitivity (SEN) and specificity (SPEC). Filters: the filters with odour of the larvae of *O.eremita*; L13, L16: larvae recorded in 2013 and 2016; F+R: frass and remains of adults; FC: larvae of flower chafers; FB: Forcella Buana; SV: San Vito.

Target	Area	Overall accuracy			Accuracy without handler correction			Handler contribution	
		ACC (%)	SEN (%)	SPEC (%)	ACC (%)	SEN (%)	SPE (%)	ACC (%)	SPE (%)
Filters		92.64	83.73	92.66	80.81	83.73	80,07	11,83	12,59
L13, L16, F+R	FB	66.67	39.13	77.05	58.33	39.13	65.57	-	-
	SV	75.00	70.00	76.31	58.33	70.00	55.26	-	-
Mean		70.83	54.56	76.68	58.33	54.56	60.41	12.50	16.27
L13, L16	FB	75.00	62.50	76.31	66.67	62.50	67.10	-	-
	SV	70.83	75.00	70.45	54.17	75.00	52.27	-	-
Mean		72.91	68.75	73.38	60.42	68.75	59.68	12.49	13.70
FC	FB	-	21.87	-	-	-	-	-	-
	SV	-	35.71	-	-	-	-	-	-
Mean		-	28.79	-	-	-	-	-	-

Preliminary discrimination test

The discrimination tests with larvae of *Oryctes* (*Oryctes*) *nasicornis* and *Gnorimus variabilis* showed similar results. Teseo correctly exclusively signalled the larvae of *O. eremita* and

Figure 5. Teseo signalling the target to the handler: **A** sitting beside a tree containing the target, barking and looking at scent source and handler **B** pointing the target, barking and looking at the handler **C** scratching the trunk and barking (Photos A and B by Emilia Capogna, photo C by Sönke Hardersen).

sometimes showed some faint reactions to the larvae of the other species (e.g. sitting beside the source of the odour or barking weakly). After 2 or 3 repetitions of the tests, Teseo showed no reactions to the larvae of *O. nasicornis* and *G. variabilis*. However, it was noticed that during a few of the training sessions following these discrimination tests, the dog committed a higher rate of errors. Nevertheless, after a few training sessions, Teseo recovered his usual level of accuracy. For this reason it was decided to stop these tests.

Measurement of accuracy

When working with nitrocellulose filters, Teseo showed an accuracy of 93%, a sensitivity of 84% and a specificity of 93% (Table 8). When searching trees with cavities occupied by *O. eremita*, accuracy of 71%, sensitivity of 55% and specificity of 77% were calculated by taking into consideration the presence of *O. eremita* live larvae, frass and remains of adults (Table 8). If the presence of frass and remains of adults were not considered, the accuracy was 73%, the sensitivity was 69% and the specificity was 73% (Table 8). When testing whether Teseo was also able to signal the presence of larvae of other species of flower chafers, the dog showed a sensitivity of 29% (Table 8). The direct contributions of the handler to the accuracy and specificity amount to 12% and 13% respectively for the work with filters and 13% and 14% with larvae (Table 8).

Efficiency

The total time spent by the two operators to investigate 149 trees by wood mould sampling amounted to 198 hours (11,880 minutes) (48 trees in 96 hours in San Vito and 101 trees in 138 hours in Forcella Buana). The total time spent by the two operators to investigate 132 trees with Teseo amounted to 855 minutes (48 trees in 376 minutes in San Vito and 84 trees in 479 minutes in Forcella Buana). The mean overall time spent by two operators per tree for wood mould sampling and with the dog amounted respectively to about 80 minutes and to 6 minutes and 50 seconds.

Discussion

The protocol developed to train the conservation detection dog in the MIPP project was successful in teaching the dog the specific task required, i.e. to find larvae of the saproxylic beetle *O. eremita* living inside hollow trees. Therefore the results showed that this rare and elusive beetle can be monitored with the aid of a trained dog. These results are in line with research on other animal species living in wood or in burrows (Brooks et al. 2003, Hoyer-Tomiczek et al. 2016, Nielsen et al. 2016) that were successfully detected with the aid of dogs. In the case of Teseo, the results obtained for accuracy, sensitivity and specificity in natural conditions are high and close to the ones obtained with nitrocellulose filters with the smell of the larvae of *O. eremita* in controlled conditions (Table 8). This supports the fact that the training plan, which involved the use of filters, was well suited for the project aim. The filters were important because they minimised the number of larvae employed for the training. For the trainer, the use of filters was much easier than using live larvae during the training sessions. A further important point is that the dog showed a high sensitivity to the target odour (i.e. the smell of larvae of *O. eremita*) and distinguished reliably between trees harbouring the larvae and trees without them (Table 8).

The highest values were obtained if only larvae were considered as the target (i.e. excluding frass and remains) (Table 8) and this is a further indication that Teseo only signalled the presence of larvae. Similar levels of accuracy were reported by Hoyer-Tomiczek et al. (2016) for field tests on an alien longhorn beetle. It is important to point out that not all the trees in which frass and remains have been found were also colonised by larvae; only the larvae of *Osmoderma* reliably indicate the presence of the species in a tree.

Confirmation that the dog has been successfully imprinted is also provided by the results of the preliminary discrimination test and by the low sensitivity in signalling larvae of other flower chafers species which often share the same cavity with *O. eremita*. In particular, Teseo showed a much lower sensitivity to larvae of flower chafers (29%) when compared to the larvae of *O. eremita* (69%). This result is similar to Brooks et al. (2003), who found that trained dogs falsely signalled termites in 25% of tests when termite-damaged wood without termites was presented. In contrast, Lin et al. (2011) reported false positives in only 4% of tests when working with ants and Pfiester et al. (2008) found false positive rate of 3% on bed bug faeces.

These results on accuracy and sensitivity of Teseo are consistent with those obtained in other studies involving dogs in the search for saproxylic beetles. For example, a sensitivity of 78% was obtained for dogs trained to find the red palm weevil, *Rhynchophorus ferrugineus* (Suma et al. 2014) and Hoyer-Tomiczek et al. (2016) obtained accuracy values between 81% and 94% for larvae of a xylophagous longhorn beetle. They also showed that dogs had a lower accuracy when searching for wood shaving and frass without larvae of the target species, as was also observed for Teseo.

The difference between the values of accuracy measured with nitrocellulose filters and larvae may depend on several factors. In general, working in a natural setting with live targets is certainly more complicated than searching for filters placed by operators. Other studies have also shown that sensitivity was lower under realistic conditions (Hoyer-Tomiczek et al. 2016). In fact, natural populations of insects can have an non-homogeneous distribution and different trees can contain a variable number of larvae and consequently a different concentration of odour. During the fieldwork, it was obvious that, in presence of strong odour sources, the dog detected the target from several metres distance. This effect can be increased by the wind that can carry the target odour away from the source. Chambers et al. (2015) and Hoyer-Tomicek et al (2016) also reported that the scent of species living in trees was transported by wind. These two variables (high concentration of target odour and wind) can confound the results and lead to signalling of trees which do not contain the target, but which are close to trees with larvae of *O. eremita*. Similarly Chambers et al. (2015) and Hoyer-Tomicek et al. (2016) concluded that wind can lead to signalling of the incorrect tree.

A further important point to consider is which factors increase the level of fatigue in the dog and consequently decrease its reliability (e.g. DeShon et al. 2016). For example, trees can be distant from each other, the vegetation can be impervious and scheduled searches might need to be conducted under sub-optimum weather conditions (e.g. high temperatures or rain).

Precautions should be taken to minimise the effects of these factors, such as: i) carry out some preliminary surveys to allow the dog to become familiar with the working area; in fact, it is well known that accuracy measurements in new areas can initially be low and can increase in later surveys (Wallner and Ellis 1976); ii) choose the most suitable time of the day for the activity of the dog, (e.g. avoid the hours with the highest temperatures, rainy days and strong wind) and iii) pay attention to the level of fatigue in the dog, decreasing the duration of the working sessions and increasing the length of the breaks if necessary or even interrupting the daily work if the dog does not show an appropriate level of concentration.

The lower accuracy measured on trees colonised by larvae of *O. eremita* may also depend on factors related to the detection probability (i.e. the probability to detect the larvae in a tree, if present) of the wood mould sampling method. In Forcella Buana and San Vito, results obtained by wood mould sampling were used to validate the accuracy, sensitivity and specificity of the signalling of Teseo. However, Chiari et al. (2014) showed that the detection probability for wood mould sampling can vary between 34% and 50%. This means that a high percentage of trees harbouring *O. eremita* were

not correctly identified by wood mould sampling. In these cases, the complete signalling by the dog was incorrectly counted as errors (false signalling).

Another very important factor to take into account is the relationship between the dog and the handler (cf. Beebe et al. 2016). A handler who correctly understands the dog's behaviour during fieldwork can increase the overall accuracy by more than 10%. Although the improvement of communication between the dog and handler can be obtained through specific double blind training sessions, it is clear that differences exist in the searching ability of different dog and handler teams (Hoyer-Tomicek et al. 2016).

Comparison between the methods: conservation detection dog and wood mould sampling

The results of the tests carried out demonstrated that the use of the CDD was a better method to detect larvae of *O. eremita* when compared to WMS. In fact, the dog showed an overall probability of detecting colonised trees in the area of Forcella Buana and San Vito of 73%, that is higher than the detection probability with WMS (34–50%) in the same areas (Chiari et al. 2014). Furthermore, the use of a dog was much less time-consuming than WMS. The average duration needed to examine a single tree by 2 operators was about 80 minutes by WMS and 6 minutes 50 seconds by CDD, respectively. This means that the dog, during a day with good weather conditions, can complete up to 4 work sessions lasting 50 minutes each (with appropriate breaks), for a total of about 60 trees. Thus, the dog can find larvae of *O. eremita* with high accuracy employing less than 1/10 of the time for WMS. In addition, the searches with the dog completely eliminate the risk of harming larvae, adults or other species living in the hollow trees.

Protocols, materials and equipment

For the base training and the imprint phase, a 3m training leash was used. Subsequently, when the dog had learned to pay attention to the trainer when he was wearing the harness, no leash was used.

Similarly, the leash was used during the initial conditioning with the clicker: conditioning sessions, lasting 5 minutes, were carried out, giving the dog small pieces of tasty food (different to that used for rewarding the dog after correct signalling) as a reward simultaneously with the click. After the dog had well learned to associate the sound of the click with the food, daily sessions (up to 15 minutes) were carried out without the leash. Metallic clickers were preferable as they were more resistant and produce a louder sound. As a reward for a target detected successfully, small pieces of chicken sausage were used. Disposable latex gloves should be used when handling filters or larvae to avoid transferring the target odour to the hands of the trainer, which might confuse the dog.

Constraints, spatial validity and possible interferences

To avoid interferences in the field during training or monitoring sessions:

- There should be as few people as possible and it would be preferable if the dog was familiar with all the people present.
- The field assistant must stay distant and out of the trajectory of the dog-handler team while working in order to avoid creating disturbance.
- If possible, working should be avoided during rainy and windy days.
- If it is necessary to work during periods with very high temperatures, the hottest hours of the day should be avoided and it is suggested that work should be undertaken early in the morning or late in the afternoon.

When trees are close to each other and in dense woods, it was observed that the dog can become confused as the target odour can apparently move from a source tree. In these cases, the dog can perceive the smell beside another tree and this effect is increased by the wind. When these conditions occur, it might be better to work on groups of trees rather than on individual trees.

It was noticed that Teseo performed better when carrying out a maximum of 5 training sessions per week, alternated with 2 days of rest. When the dog was fully trained (i.e. he reached the expected level of accuracy), the training rate could be reduced to 2 or 3 sessions per week (i.e. maintenance training). However, before working in the field, the rate of training should be increased again to 5 times per week. It would be necessary to start at least 6 weeks earlier and to gradually increase the following parameters: the number of weekly training sessions, the number of daily sessions, the number of trees examined (both with and without target) and the general complexity of the sessions. It is recommended to carry out some training sessions in which only one target is used (in the last tree surveyed during the session) in order to get the dog used to working in areas with low population density of the target species (or where the target species might not be present).

Conclusions

A conservation detection dog is a powerful tool for locating *O. eremita* and these results can be useful for the other related European species of *Osmoderma* (Audisio et al. 2007, 2009, Zauli et al. 2016) and in general for other saproxylic insects. In fact, the use of a trained dog is a fast, accurate and non-invasive method that allows the detection of a target species in an area and to identify the colonised trees; this means that a CDD can locate new populations, can confirm the presence of the target species and can assist in the mapping of the distribution of colonised trees in an area, accurately and efficiently. Furthermore, monitoring by means of dogs can be carried out in the same area repeatedly and this allows the detection probability for this method to be obtained. These

values can then be compared with detection probabilities for other methods employed for the same species (e.g. wood mould sampling). Repeating the search with a CDD in the same area but in subsequent years would allow changes in the number and distribution of trees occupied by the beetle to be monitored.

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